

CLAIMS

We claim:

1. A method for the formation of embryoid bodies, comprising (a)
5 inoculating a culture vessel with a culture of undifferentiated embryonic stem cells,
wherein said culture vessel contains a medium suitable for inducing embryoid body
formation; and (b) incubating said culture vessel while subjecting it to shaking.
2. The method of claim 1, wherein said shaking comprises rotary
10 shaking at a rate from about 60 to about 200 RPM.
3. The method of claim 1, wherein said undifferentiated embryonic
stem cells are of mammalian origin.
- 15 4. The method of claim 3, wherein said undifferentiated embryonic
stem cells are of murine origin.
5. The method of claim 3, wherein said undifferentiated embryonic
stem cells are of human origin.
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6. A method for differentiating embryonic stem cells, comprising: (a)
inoculating a culture vessel with a culture of undifferentiated embryonic stem cells,
wherein said culture vessel contains a medium suitable for inducing embryoid body
formation; (b) incubating said culture vessel while subjecting it to shaking; (c)
25 continuing said incubating until embryoid bodies are produced; (d) dissociating the
resulting embryoid bodies; (e) inoculating the dissociated embryoid bodies into a
medium suitable for accomplishing further differentiation; and (f) culturing said
dissociated embryoid bodies to a later differentiation state.
- 30 7. The method of claim 6, wherein said shaking comprises rotary
shaking at a rate from about 60 to about 200 RPM.
8. The method of claim 6, wherein said dissociating step comprises
trypsinization of the embryoid bodies.

9. The method of claim 6, wherein said medium suitable for accomplishing further differentiation is comprised of at least one molecule that accomplishes final differentiation of said embryoid bodies.

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10. The method of claim 9, wherein said culturing step results in the formation of a differentiated embryonic stem cell.

11. The method of claim 10 wherein said differentiated stem cell is
10 selected from the group consisting of: macrophages, hepatocytes, pancreatic cells, neural cells, cardiac cells, smooth muscle cells, pancreatic tissue, liver tissue, smooth muscle tissue, striated muscle tissue, cardiac muscle tissue, bone tissue, bone marrow tissue, bone spongy tissue, cartilage tissue, liver tissue, pancreas tissue, pancreatic ductal tissue, spleen tissue, thymus tissue, tonsil tissue, Peyer's
15 patch tissue, lymph nodes tissue, thyroid tissue, epidermis tissue, dermis tissue, subcutaneous tissue, heart tissue, lung tissue, vascular tissue, endothelial tissue, blood cells, bladder tissue, kidney tissue, digestive tract tissue, esophagus tissue, stomach tissue, small intestine tissue, large intestine tissue, adipose tissue, uterus tissue, eye tissue, lung tissue, testicular tissue, ovarian tissue, prostate tissue,
20 connective tissue, endocrine tissue, mesentery tissue, fetal tissue and umbilical tissue.

12. The method of claim 6, wherein said method further comprises: (g) contacting a differentiated cell produced by said culturing step with a therapeutic
25 agent.

13. The method of claim 6, wherein said method further comprises: (g) using a cell produced by said culturing step in a cell replacement therapy.

14. The method of claim 6, wherein said method further comprises: (g) using a cell produced by said culturing step to produce a tissue.
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15. The method of claim 6, wherein said method further comprises: (g) determining the gene expression of the cells or embryoid bodies in the culture in at least one of (a) through (e).

5 16. The method of claim 6, wherein said method comprises: (g) determining the gene expression in a differentiated cell produced by said culturing step and in an embryoid body produced by said incubating step.

10 17. A cellular composition of embryoid bodies, wherein at least 80% of cells comprising said embryoid bodies have a diameter from about 13 to about 15 microns.

15 18. The composition of claim 12 wherein at least 95% of cells comprising said embryoid bodies are viable.

19. The composition of claim 12 wherein at least 10% of cells comprising said embryoid bodies express CD34.

20 20. A cellular composition of embryoid bodies, wherein at least 20% of cells comprising said embryoid bodies express FLK-1.